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SOME *IN VITRO* EFFECTS OF *HERPESTIS MONNIERA* LINN ON THE RESPIRATION OF RAT BRAIN*.

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[Received for publication, April 7, 1961.]

THE plant *Herpestis monniera* (N.O. *Scrophularineae*) indigenous to India has been claimed to be a potent nervine tonic. It is being extensively used for various nervous disorders, such as insanity, epilepsy, hysteria, etc., in the Ayurvedic system of medicine (Chopra, 1938).

Bose and Bose (1931) claimed that the plant contained a very small amount of alkaloid 'brahmine' which was found to be highly toxic in frogs and guinea-pigs. Sastry, Dhalla and Malhotra (1959) isolated hersaponin and D-mannitol and also confirmed the presence of alkaloids. Malhotra and Das (1959) reported that the total extract of *Herpestis monniera* has cardiotonic, vasoconstrictor, sedative and neuro-muscular blocking actions. It was also found that hersaponin has cardiotonic action in frogs, sedative action in albino rats and guinea-pigs, and spasmodic action on certain smooth muscles. Recently, Malhotra, Das and Dhalla (1960) have evaluated the sedative action of hersaponin in mice and have established its hypnotic potentiating activity using barbiturates and ethanol. Hersaponin did not protect rats against electroshock and mice against metrazol seizures. It produced hypothermia in mice and reduced the amphetamine toxicity in aggregated mice but had no effect on metrazol toxicity. It has also been suggested that the central action of hersaponin simulate, those of the known tranquillizers—reserpine and chlorpromazine, having some properties of each. Prakash and Sirsi (1960) have established the psychotropic action of *Herpestis monniera* in facilitating the 'motor learning' in rats. Subsequently, Aithal and Sirsi (1961) have shown that the alcoholic extract of the drug exhibited tranquillizing effect on rats and dogs. The present paper deals with the effect of total extract, alkaloidal fraction and hersaponin isolated from *Herpestis monniera* on the respiratory activity *in vitro* of rat brain.

MATERIALS AND METHODS.

Albino rats of either sex weighing 120 g. to 150 g. were used. The animals were killed by decapitation and the brain was immediately removed, weighed, washed and transferred to a previously chilled glass tissue grinder containing Krebs Ringer phosphate buffer pH 7·3 (Umbreit, Burris and Stauffer, 1957). The brain homogenate was diluted with buffer so that each c.c. of homogenate contained 100 mg. of the fresh tissue.

Oxygen consumption was measured at 10 minute intervals by conventional Warburg manometric technique. The gas phase was air and the temperature of the

* Studies carried out under 'Drug Research Unit' with grant from Indian Council of Medical Research.

bath was 38°C. Each reaction vessel contained 1 c.c. of the rat brain homogenate and solvent control (0·3 c.c.) with or without drug. In those experiments where substrates were used, 0·3 c.c. of glucose (0·33 M), or 0·3 c.c. of sodium glutamate (2·0 M), or 0·3 c.c. of sodium succinate (0·33 M), all dissolved in phosphate buffer were added. In some experiments, 0·3 c.c. of 10^{-5}M lysergic acid diethyl amide (LSD 25) or 0·3 c.c. of 10^{-2}M 5-hydroxytryptamine (5-HT), in phosphate buffer was added. The total volume of the fluid in the flask was always made up to 3 c.c. with phosphate buffer. The central well of the flask contained 0·2 c.c. of 20 per cent KOH absorbed on filter paper.

The drug was tipped in from the side arm at zero time after fifteen minutes equilibration. All the measurements were performed in triplicate and the rate of oxygen uptake, Q_{O_2} for the first sixty minutes was calculated as μl. of oxygen per hour per 100 mg. of the brain tissue (Shatzko and Sim, 1959; Starbuck and Heim, 1959). All the concentrations of drug refer to the final concentrations in reaction vessel.

The drugs used were *Herpestis monniera* extract and its constituents isolated as given below :—

Total extract : (Yield 5 per cent).—The hot alcoholic extract was concentrated to remove the solvent and freed from chlorophyll and salts.

Alkaloidal fraction : (Yield 0·05 per cent).—Above extract was digested with 1 per cent hot hydrochloric acid. The acid solution was freed from acidic material and the bases were extracted with chloroform after making alkaline with ammonium hydroxide.

Hersaponin : (Yield 0·02 per cent).—It was isolated by the method described earlier (Sastry, Dhalla and Malhotra, 1959).

The total extract was taken in phosphate buffer, while the alkaloidal fraction and hersaponin were dissolved in phosphate buffer containing 1 per cent propylene glycol which also served as control. The effect of solvent control was found to be insignificant and hence omitted from the text.

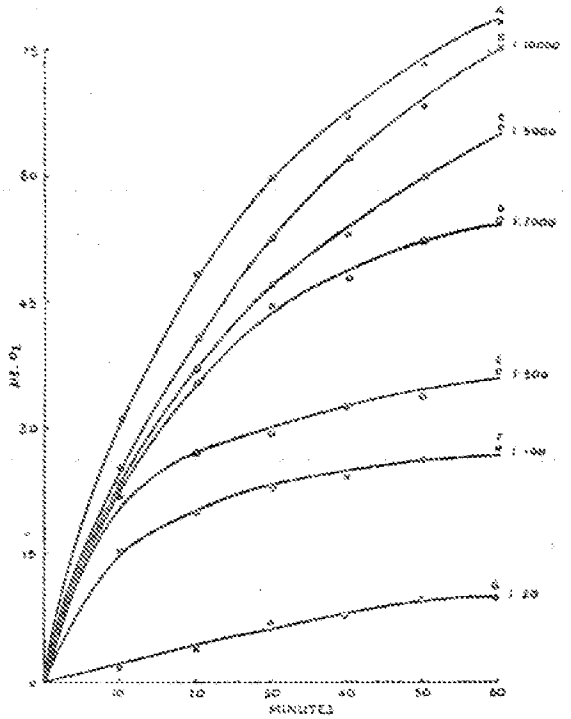
Results.

The rates of oxygen consumption at different intervals by 100 mg. of the brain homogenate in phosphate buffer in the presence of various concentrations of total extract and alkaloidal fraction of *Herpestis monniera* are given in Graphs 1 and 2 respectively. Each reading is the average of 9 to 21 reaction vessels in several experiments for the same concentration of the drug and is within the variable range of ± 5. The effect of various concentrations of the total extract and other constituents of *Herpestis monniera* on the respiration of rat brain are given in Table I. The values show the oxygen uptake as μl. of oxygen, per hour per 100 mg. of the brain tissue and are the average of the number of experiments mentioned against each concentration.

In each experiment the same homogenate was used for the oxygen uptake with or without the drug. Variations in the respiratory rate have been calculated separately

GRAPH 1.

The effect of different concentrations of total extract of Herpestis monniera on oxygen uptake of rat brain homogenate in phosphate buffer.



A—solvent control; B, C, D, E, F and G—different concentrations of total extract.

in each experiment. Though the rate of oxygen uptake in the control flasks varied in each experiment, the percentage effect of the drugs showed very little variation for the same concentration. All the experimental results have been subjected to the statistical analysis and the standard error calculated is based on the percentage effect.

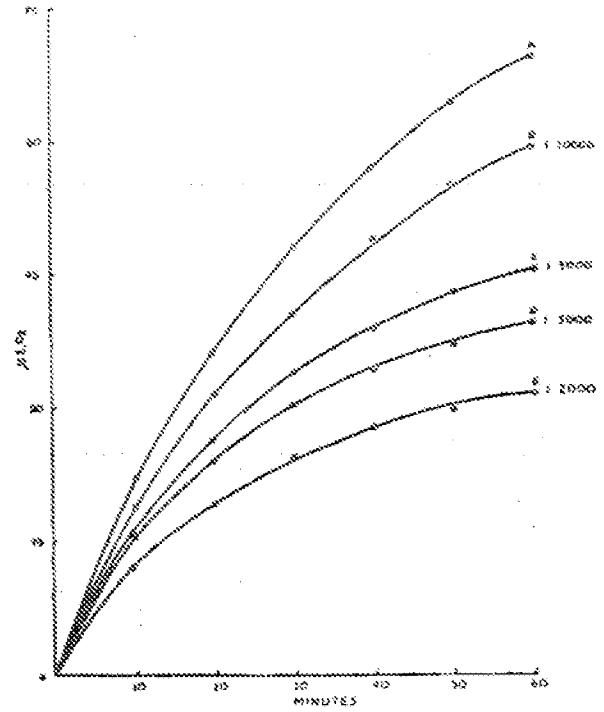
Table I shows that both the total extract and alkaloidal fraction had varying degrees of inhibitory action on the oxidative process. It is quite interesting to note that hersaponin, even in higher concentrations had no considerable depressing action on the respiration of brain *in vitro*.

ED_{50} (with 10/20 confidence limits) calculated by the method of Litchfield and Wilcoxon (1949) was found to be 1 : 446 (1 : 1,020—1 : 229) and 1 : 2,291 (1 : 4,000—1 : 1,348) for the total extract and alkaloidal fraction, respectively. A comparison of the ED_{50} shows that the effect of the alkaloidal fraction was five times more than that of the total extract. On the other hand, in the same concentration (1 : 500), hersaponin was about one third as effective as the total extract.

The oxygen uptake by 100 mg. of the rat brain homogenate in the presence of glucose (0·03 M), sodium glutamate (0·20 M), and sodium succinate (0·03 M) as

GRAPH 2.

The effect of different concentrations of alkaloidal fraction from Herpestis monniera on oxygen uptake of rat brain homogenate in phosphate buffer containing 1 per cent propylene glycol.



A—solvent control ; B, C, D and E—different concentrations of alkaloidal fractions.

substrates with or without the alkaloidal fraction of *Herpestis monniera* is given in Table II. The percentage inhibition was least in sodium glutamate, moderate in sodium succinate, and marked in glucose. The approximate ratio of the inhibitory effect of alkaloidal fraction in their presence is 2 : 3 : 6, respectively.

The influence of LSD-25 (10^{-6}M) and 5-HT (10^{-6}M) on the respiration with or without the alkaloidal fraction and hersaponin has been recorded in Table III. It will be seen from the table that the inhibitory effect produced by the addition of LSD-25 to the Warburg vessel containing hersaponin was less than that of either LSD-25 or hersaponin. There was no change in the action of alkaloidal fraction in the presence of LSD-25. 5-HT, on the other hand, potentiated the action of hersaponin considerably, even though it had individually inhibitory action on the respiration of brain *in vitro*, while it did not exhibit any influence on the action of alkaloidal fraction.

DISCUSSION.

The present studies show that the total extract of *Herpestis monniera* had inhibitory action on the respiration of rat brain homogenate. The action was found to be mainly due to its alkaloidal content. The inhibitory effect of the alkaloidal

TABLE I.

Effect of total extract of Herpestis monniera *and its constituents on the oxygen uptake of rat brain homogenate.*

Concentration of drug.		Qo_2 without drug*.	Qo_2 with drug*.	Per cent inhibition (with S. E.)
A. *Total extract :*				
1 : 10,000	(3)†	78·9	68·7	5·8 ± 0·5
1 : 5,000	(3)	78·9	62·1	14·7 ± 0·3
1 : 2,000	(4)	74·8	49·7	30·8 ± 2·0
1 : 500	(4)	76·2	37·5	52·4 ± 1·0
1 : 100	(10)	78·8	25·5	67·6 ± 1·0
1 : 20	(5)	76·3	9·2	87·1 ± 1·2
B. *Alkaloidal fraction :*				
1 : 10,000	(3)	77·9	66·9	13·6 ± 2·0
1 : 5,000	(4)	67·6	44·6	32·2 ± 0·9
1 : 3,000	(6)	68·9	38·3	43·2 ± 1·8
1 : 2,000	(5)	76·1	32·3	55·1 ± 1·8
C. *Hersaponin :*				
1 : 5,000	(4)	74·9	72·1	3·8 ± 0·5
1 : 1,000	(5)	76·7	70·8	7·9 ± 1·8
1 : 500	(8)	71·2	58·7	16·1 ± 1·2

*Calculated as μl./hr./100 mg. of brain tissue.

†Figures within brackets show the number of experiments carried out in each study.

TABLE II.

Effect of alkaloidal fraction (5×10^{-4}) with or without various substrates-glucose, sodium glutamate and sodium succinate on the respiration of rat brain.

Substrate.		Qo_2 without drug*.	Qo_2 with drug*.	Per cent inhibition. (with S.E.).
Control	(5) †	74·2	33·7	54·5 ± 1·5
Glucose (0·03M)	(5)	80·6	40·2	50·1 ± 1·7
Sodium glutamate (0·02M)	(4)	83·4	67·9	18·6 ± 1·1
Sodium succinate (0·03M)	(4)	110·2	80·1	27·4 ± 2·3

*Calculated as μl./hr./100 mg. of brain tissue.

†Figures within brackets show the number of experiments carried out in each study.

fraction on the oxidative process could not be significantly influenced by 5-HT or LSD-25. However, there was slight potentiation of action in each case. From our study of the influence of substrates on the action of alkaloidal fraction, it is quite apparent that both sodium glutamate and sodium succinate partially block its inhibitory effect. It is, therefore, suggested that the alkaloidal fraction is suppressing the enzyme system and the action might be localized somewhere in the citric acid cycle. Further work is being carried out to elucidate the mechanism of action of alkaloidal fraction *in vivo*.

Malhotra, Das and Dhalla (1960) have reported that the hypnotic potentiating action of hersaponin was inhibited on pretreatment with LSD-25 and also have

TABLE III.

Effect of lysergic acid diethyl amide and 5-hydroxytryptamine with or without alkaloidal fraction (5×10^{-4}) and hersaponin (1×10^{-3}) on the respiration of rat brain.

Drug.	Q_{O_2} in μl./hr./100 mg. of brain tissue*.	Per cent inhibition. (with S.E.).	Probability†.
Control	74·3	..	..
LSD-25 (10^{-8}M)	66·8	11·3 ± 0·4	..
5-HT (10^{-8}M)	58·3	21·9 ± 0·8	..
Alkaloidal fraction	34·8	53·7 ± 1·2	..
Alkaloidal fraction *plus* LSD-25 (10^{-8}M)	31·5	57·8 ± 1·4	P > 0·05
Alkaloidal fraction *plus* 5-HT (10^{-8}M)	32·0	56·8 ± 1·2	P > 0·05
Hersaponin	67·1	10·0 ± 0·2	..
Hersaponin *plus* LSD-25 (10^{-8}M)	70·2	5·3 ± 0·1	P < 0·05
Hersaponin *plus* 5-HT (10^{-8}M)	48·9	34·7 ± 1·0	P < 0·001

*Four experiments were carried out in each study. †Probability (P) of no significance calculated by 't' test.

suggested that release of 5-HT might be one of the ways in which hersaponin acts. From our observations, it is evident that LSD-25 reduced the respiratory inhibition caused by hersaponin and is in agreement with *in vivo* results. Potentiation caused by 5-HT *in vitro* on the inhibiting action of hersaponin lends further support to the suggested mechanism *in vivo*. Since hersaponin exerted very slight respiratory inhibition *in vitro*, it is suggested that the marked sedative and hypnotic potentiating effects of hersaponin might be due to the formation of some metabolic product in the body.

Substances, such as 2, 4-Dinitrophenol which depress the formation of high energy phosphate bond without markedly affecting oxygen consumption are known as uncoupling agents. Reserpine and chlorpromazine are also found to inhibit oxidative phosphorylation (Kirpekar and Lewis, 1960). As this very new drug 'hersaponin' has been shown to possess great potentialities with special reference to central nervous system, it would therefore, be interesting to study its influence on uncoupling oxidative phosphorylation.

SUMMARY.

1. The effect of the total extract of *Herpestis monniera* and its constituents, namely alkaloidal fraction and hersaponin on the oxygen uptake of the rat brain homogenate has been studied by Warburg technique.

2. Total extract and alkaloidal fraction inhibited the respiration of brain tissue to a varying degree. ED_{50} were found to be 1 : 446 and 1 : 2,291, respectively. Hersaponin had a mild inhibitory action.

3. In the presence of glucose, sodium glutamate and sodium succinate as substrates, the alkaloidal fraction in concentration of 5×10^{-4} exhibited 50·1, 18·6 and 27·4 per cent inhibition, respectively.

4. LSD-25 and 5-HT could not influence the inhibitory effect of alkaloidal fraction, whereas with hersaponin the action was partially reduced by LSD-25 and potentiated by 5-HT.

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